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METABOLISM OF *m*-TYROSINE IN THE RAT

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Abstract—In order to study the metabolism of *m*-tyrosine, ^{14}C-labeled *m*-hydroxyphenyl compounds were synthesized. After intraperitoneal administration of DL-[2-^{14}C] *m*-tyrosine, most of the radioactivity was eliminated via the urinary route. The radiolabeled metabolites have been investigated quantitatively. The major metabolites found were: *m*-tyramine, *m*-hydroxyphenylacetic acid, *m*-hydroxyphenylpyruvic acid and *m*-hydroxymandelic acid. In addition, two unidentified conjugates of *m*-hydroxyphenylacetic acid and *m*-tyramine were found. The metabolic fates of the D- and L-isomers of *m*-tyrosine were investigated comparatively. Results from the *in vivo* and *in vitro* experiments on the metabolism of [^{14}C]*m*-hydroxyphenyl compounds demonstrate that decarboxylation is the key step in the metabolism of *m*-tyrosine, and that liver, kidney and brain have the enzymes necessary to account for the major *m*-tyrosine metabolites.

In recent years, there has been great interest in *m*-hydroxyphenyl compounds owing to the demonstration of these compounds in mammalian urine and tissues [1–6]. Furthermore, investigators from many laboratories have reported that 3-hydroxyphenylalanine (*m*-tyrosine) has many pharmacological actions similar to those of dopa* [7–12], which is used currently in the treatment of Parkinsonism [13, 14]. Present knowledge of the biochemistry of *m*-tyrosine and other *m*-hydroxyphenyl compounds is derived mostly from *in vitro* experiments designed to test whether they are substrates for the enzymes involved in the metabolism of catecholamines [15–18]. However, little data exist on their metabolism *in vivo*.

The development of a convenient method for the resolution of ring-substituted phenylalanines [19], the synthesis of radiolabeled *m*-tyrosine [20] and the lack of information on the metabolism of *m*-hydroxyphenyl compounds *in vivo* have prompted this investigation.

MATERIALS AND METHODS

The following were purchased from the Sigma Chemical Co., St. Louis, MO: *m*-hydroxyphenylacetic acid, FAD, pyridoxal phosphate and glutamine. 2-Oxoglutaric acid was obtained from the Nutritional Biochemicals Corp., Cleveland, OH, and *m*-methoxybenzaldehyde from Koch-Light Lab. Ltd., Colnbrook, Bucks, U. K. [^{14}C] sodium cyanide and [^{14}C] nitromethane were purchased from the New England Nuclear Corp., Boston, MA. [2-^{14}C]*m*-tyrosine and other materials were obtained as reported previously [20].

Syntheses of ^{14}C-*labeled* m-*hydroxyphenyl compounds*

Radiolabeled *m*-hydroxyphenyl acids were synthesized by modifications of the methods described by Shaw *et al.* [21], using suitable ^{14}C-labeled starting materials. All evaporation procedures were carried out at 40° *in vacuo*.

[2-^{14}C]m-*hydroxyphenylpyruvic acid.* 2-Methyl-4-(*m*-acetoxybenzal) [4-^{14}C]oxazoline-5-one (2.45 g, 0.38 μCi) was suspended in 1 N HCl (100 ml). The mixture was refluxed for 5 hr under nitrogen. The hot solution was treated with charcoal and filtered through Celite. After cooling, the filtrate was extracted (6 times) with ethyl acetate (25 ml). The combined organic phase was extracted (3 times) with 1 N $NaHCO_3$ (20 ml). The combined aqueous phase was made acidic with HCl. The product was then back extracted (6 times) into ethyl acetate (20 ml). The combined extract was dried ($MgSO_4$) and evaporated to give an oily residue. The crystalline product was obtained by dissolving the oily residue in ethyl acetate (20 ml), followed by dropwise addition of an equal volume of 1, 2-dichloroethane and storage at 4° for 3 days. The [2-^{14}C]*m*-hydroxyphenylpyruvic acid obtained had a m.p. of 164–166° (lit. m.p. 164–165° [21]) and weighed 0.95 g, corresponding to a yield of 51 per cent. The product could be stored at −20° *in vacuo* for 3 months without measurable decomposition and had a specific activity of 2.08 μCi/10 mg.

[1-^{14}C]m-*hydroxymandelonitrile. m*-Hydroxybenzaldehyde (1.22 g) and sodium bisulfite (1.26 g) were dissolved in water (12 ml) at 50°. The solution was chilled to −5° in an ice-salt bath and diethyl ether (7 ml) was added with stirring. ^{14}C-labeled sodium cyanide (0.49 g, 0.5 mCi), dissolved in cold water (2 ml), was added dropwise to the above mixture and stirring was continued for 45 min. The ether phase was saved and the aqueous phase was further extracted (4 times) with diethyl ether (7 ml). The combined ether fraction was washed (2 times) with 1 N $NaHSO_3$ (5 ml), dried ($MgSO_4$), treated with charcoal, and filtered through Celite. The filtrate was concentrated to about 5 ml and benzene was added until the solution was slightly cloudy. The mixture was stored at 4° overnight. The product collected by filtration had a m.p. of 108–109° and weighed 0.77 g, corresponding to a yield of 52 per cent. The specific activity of the product was 3.35 μCi/10 mg.

* The following abbreviations were used: dopa, 3,4-dihydroxyphenylalanine; FAD, flavin-adenine dinucleotide; Catron, β-phenyl-isopropyl hydrazine; MK-486 (Carbidopa), L-α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl)-propionic acid; NSD-1034, *N*-methyl-*N*-(3-hydroxybenzyl)-hydrazinium dihydrogen phosphate; *m*-HPPA, *m*-hydroxyphenylpyruvic acid; *m*-HMA, *m*-hydroxymandelic acid; and *m*-HPAA, *m*-hydroxyphenylacetic acid.

[*1*-^{14}C]m-*hydroxymandelic acid.* [1-^{14}C]*m*-hydroxymandelonitrile (0.39 g) was dissolved in a mixture of ethanol (0.17 ml) and diethyl ether (2 ml). Anhydrous HCl was bubbled through the solution for 2 min with cooling in ice. A yellow gum precipitated, and the reaction mixture was left at 4° for 3 hr. The solvent was discarded and the precipitate was dried over KOH *in vacuo*. The residue dissolved in 1.2 N NaOH (16 ml) was refluxed for 2 hr under nitrogen. After cooling, the mixture was adjusted to pH 7.5 with HCl and washed (4 times) with ethyl acetate (5 ml). The aqueous phase was then made acidic with HCl and the product was extracted (6 times) with ethyl acetate (5 ml). The combined ethyl acetate fraction was dried ($MgSO_4$), treated with charcoal, and filtered through Celite. The filtrate was evaporated to dryness. The residual oil was dissolved in ethyl acetate (1 ml), and cyclohexane was added until the solution was slightly cloudy. The mixture was stored at 4° overnight. The [1-^{14}C]*m*-hydroxymandelic acid obtained had a m.p. of 131° (lit. m.p. 130–131° [21]) and weighed 0.29 g, corresponding to a yield of 64 per cent. The specific activity of the product was 2.97 μCi/10 mg.

[*1*-^{14}C]m-*hydroxyphenylacetic acid.* A mixture of [1-^{14}C]*m*-hydroxymandelonitrile (0.37 g), freshly distilled hydriodic acid (15 ml) and red phosphorus (0.47 g) was refluxed overnight. After cooling, the reaction mixture was filtered through a sintered glass funnel and the filtrate was evaporated to dryness. The evaporation process was repeated (3 times) after addition of water (10 ml). The residue was dissolved in water (10 ml), treated with charcoal, and filtered through Celite. The filtrate was adjusted to pH 7.5 with NaOH and washed (3 times) with diethyl ether (10 ml). The aqueous phase was then made acidic with HCl, and the product was extracted (5 times) with diethyl ether (20 ml). The combined ether fraction was evaporated to dryness. Crystalline product was obtained by dissolving the residue in diethyl ether (5ml), followed by the addition of cyclohexane (12 ml) and storage at 4° overnight. The [1-^{14}C]*m*-hydroxyphenylacetic acid obtained had a m.p. of 128–129° (lit. m.p. 130–131° [21]) and weighed 0.27 g, corresponding to a yield of 71 per cent. The specific activity of the product was 3.16 μci/10 mg.

The syntheses of [1-^{14}C]*m*-tyramine and [1-^{14}C]-*m*-octopamine were achieved by coupling *m*-methoxybenzaldehyde with ^{14}C-labeled nitromethane [22] and ^{14}C- labeled sodium cyanide [21], respectively, followed by reduction with lithium aluminium hydride [23] and cleavage of the methyl ether with hydrochloric acid under pressure [24].

dehyde (1.36 g) was dissolved in 95 per cent ethanol (80 ml). The solution was chilled in an ice-bath to 5° and [1-^{14}C]nitromethane (0.61 g, 1 mCi) was added with stirring. Sodium hydroxide (1 g), dissolved in 95% ethanol (20 ml), was added to the above mixture over a period of 20–30 min, followed by the addition of enough water to dissolve the precipitate. The solution was then added dropwise to 4 N HCl (30 ml) at room temperature with stirring. The resulting suspension was filtered, and the precipitate was washed (3 times) with cold water (5 ml). A second batch of precipitate was recovered by evaporating the filtrate to dryness and washing the residue with cold water. The combined precipitate was dissolved in ethyl acetate (15 ml), treated with charcoal, and filtered through Celite. [1-^{14}C]*m*-methoxynitrostyrene was recovered from the filtrate after removal of ethyl acetate by evaporation. The product (sp. act. 5.75 μCi/10 mg) had a m.p. of 88–89° and weighed 1.30 g, corresponding to a yield of 75 per cent.

[*1*-^{14}C]m-*methoxyphenylethylamine*·HCl. [1-^{14}C]*m*-methoxynitrostyrene (1.30 g) was dissolved in anhydrous diethyl ether (100 ml). The solution was added to a suspension of lithium aluminium hydride (0.82 g) in anhydrous diethyl ether (150 ml) over a period of 30 min with stirring. The mixture was refluxed for 18 hr. After cooling, the excess hydride was decomposed by dropwise addition of water (0.3 ml), 5 N NaOH (0.4 ml) and water (1.2 ml). The suspension was stirred for 30 min and filtered. The filtrate was evaporated to dryness and 2 N HCl (5 ml) was added to the residue. The mixture was evaporated again to dryness and the residue was dissolved in ethanol (12 ml). The solution was added dropwise to diethyl ether (60 ml) with stirring. The [1-^{14}C]*m*-methoxyphenylethylamine·HCl collected by filtration had a m.p. of 130–131° and weighed 0.91 g, corresponding to a yield of 67 per cent. The specific activity of the product was 5.54 μCi/10 mg.

[*1*-^{14}C]m-*tyramine*·HCl. In a pressure bottle, [1-^{14}C]*m*-methoxyphenylethylamine·HCl (0.46 g) was dissolved in 12 N HCl (1.5 ml). After flushing with nitrogen, the bottle was sealed and heated at 140–150° for 4 hr in a glycerol bath. The reaction mixture was then evaporated to dryness (3 times) after adding ethanol (5 ml). The residue was dissolved in ethanol (1.5 ml) and the solution was added dropwise to diethyl ether (15 ml). [1-^{14}C]*m*-tyramine·HCl collected by filtration had a m.p. of 134–136° (lit. m.p. 134–136° [24]) and weighed 0.28 g, corresponding to a yield of 70 per cent. The specific activity of the product was 6.20 μCi/10 mg.

[*1*-^{14}C]m-*methoxymandelonitrile.* The compound was synthesized by the method described in the synthesis of [1-^{14}C]*m*-hydroxymandelonitrile, using *m*-methoxybenzaldehyde (1.36 g) as the starting material. The product in the combined ether fraction was not isolated but used directly in the next step.

[*1*-^{14}C]m-*methoxyphenylethanolamine*·HCl. Reduction of [1-^{14}C]*m*-methoxymandelonitrile with lithium aluminium hydride was carried out as described in the synthesis of [1-^{14}C]*m*-methoxyphenylethylamine·HCl. The crystalline product had a m.p. of 108–110° and weighed 1.02 g, corresponding to a yield of 50 per cent. the specific activity of the product was 4.89 μCi/10 mg.

[*1*-^{14}C]m-*octopamine*·HCl. The compound was obtained by cleavage of [1-^{14}C]*m*-methoxyphenylethanolamine·HCl (0.05 g) with hydrochloric acid under pressure, as described in the synthesis of [1-^{14}C]*m*-tyramine·HCl. The [1-^{14}C]*m*-octopamine·HCl obtained had a m.p. of 154–155° and weighed 0.22 g, corresponding to a yield of 57 per cent. The specific activity of the product was 4.46 μCi/10 mg.

Identification and radiochemical purity of ^{14}C-*labeled* m-*hydroxyphenyl compounds*

The identification of the ^{14}C-labeled *m*-hydroxyphenyl compounds was confirmed by infrared spectroscopy and by agreement of melting points with those reported

Table 1. Ion exchange chromatography of *m*-hydroxyphenyl acids*

	Elution time (min)
m-Hydroxyphenylpyruvic acid	14
m-Hydroxymandelic acid	23
m-Hydroxyphenylacetic acid	71
m-Tyrosine	111

* Elution was performed with 0.2 M sodium citrate buffer at pH 3.28 for 30 min, followed by pH 4.25 buffer at 68 ml/hr and 57° on a 50-cm column (AA-15 resin), which was coupled at the outlet to a fraction collector. Two-min fractions were collected and examined by color reaction with diazotized *p*-nitroaniline [25].

in the literature. Ion exchange and thin-layer chromatography (Tables 1 and 2), using commercially available authentic compounds as standards, showed that the synthesized compounds were over 98 per cent radiochemically pure.

Collection of urine

Compounds were administered to male Sprague–Dawley rats (85–100 g) by intraperitoneal injection, using either 0.9% NaCl or dilute HCl as a vehicle. The animals were placed in stainless steel metabolic cages. Urine was collected over 2 N HCl (1 ml), filtered through Celite, and stored at 4° until used.

Isolation and identification of ^{14}C-labeled metabolites

Urine samples (1–2 ml) were analyzed for acidic and neutral ^{14}C-labeled metabolites by ion exchange chromatography (amino acid analyzer, Beckman model 120B). Experimental details and elution times of authentic compounds are given in Table 1. Radioactivity was quantitated by liquid scintillation counting with a Nuclear-Chicago Mark I scintillation spectrometer. In some experiments, the radioactive fractions were freeze-dried. The residues were extracted with a small volume of 0.01 N HCl and identified by co-chromatography with authentic compounds on thin-layer plates (Table 2).

Table 2. Thin-layer chromatography of *m*-hydroxyphenyl compounds*

	R_f values	
	A	B
m-Tyrosine	0.08	0.00
m-Octopamine	0.42	0.00
m-Tyramine	0.58	0.00
m-Hydroxymandelic acid	0.10	0.18
m-Hydroxyphenylpyruvic acid	0.13	0.36
m-Hydroxyphenylacetic acid	0.07	0.52

* Thin-layer chromatography was performed on precoated silica gel G plates with the following solvent systems: (A) tert-amyl alcohol–ammonium hydroxide (4:1) and (B) benzene–propionic acid–water (2:2:1) (organic phase). The *m*-hydroxyphenyl compounds were visualized by spraying with diazotized *p*-nitroaniline reagent [25].

^{14}C-labeled basic metabolites from urine samples were isolated and identified as follows. Urine was adjusted to pH 6–7 and applied to an Amberlite CG-50 (Sodium form) column (0.5 × 4.0 cm). The column was washed with water (5 ml) and the basic metabolites were eluted with 2 N HCl (3 ml). The sample was freeze-dried. The residue was extracted with a small volume of 0.01 N HCl and analyzed by thin-layer chromatography.

Distribution of DL-[2-^{14}C]m-*tyrosine in rat tissues*

At a given time after administration, the rats were decapitated. Adrenal, brain, heart, kidney, liver and spleen were removed, weighed and homogenized in 0.4 N $HClO_4$ (15 ml for liver, 5 ml for all other organs). The homogenate was centrifuged at 18,000 *g* for 10 min. The pellet was discarded and the radioactivity in the supernatant fraction was quantitated by liquid scintillation counting.

In vitro *experiments*

Brain, kidney or liver was homogenized with 4 vol. of 0.25 M sucrose. All manipulations were carried out at 0–4° except in the course of incubation. Incubation was performed in a shaking water bath for 30 min at 37°. Ion exchange chromatography was performed with 2 ml resin in a small column. ^{14}C-labeled products were quantitated by liquid scintillation counting. In some experiments, the reaction products isolated by ion exchange chromatography were lyophilized. They were then identified by co-chromatography with authentic compounds (Tables 1 and 2).

(*a*) *Transamination of* L-m-*tyrosine to* m-*hydroxyphenylpyruvic acid.* The complete reaction mixture contained the following components (in μmoles) in a final volume of 1.0 ml: potassium phosphate, pH 7.7, 100; MK-486 (Carbidopa, dopa decarboxylase inhibitor), 0.01; pyridoxal phosphate, 0.05; 2-oxoglutaric acid, 0.5; L-[2-^{14}C]*m*-tyrosine, 1.5 (containing 80,000 cpm); and 0.1 ml of tissue homogenate. Samples without pyridoxal phosphate and 2-oxoglutaric acid served as controls. After incubation, the reaction was stopped with 0.5 ml of 2 N HCl. The precipitate was removed by centrifugation, and 0.5 ml of the supernatant fraction was applied to a Dowex AG50-X4 (H^+) column. The radiolabeled *m*-hydroxyphenylpyruvic acid formed was eluted with water (2 ml).

(*b*) *Transamination of* m-*hydroxyphenylpyruvic acid to* m-*tyrosine.* The assay procedure in this experiment was similar to that described in (a), except that glutamine replaced 2-oxoglutaric acid and [2-^{14}C]*m*-hydroxyphenylpyruvic acid (5 μmoles, containing 58,000 cpm) replaced L-[2-^{14}C]*m*-tyrosine in the reaction mixture. The Dowex AG50 column was washed with water (10 ml), and radiolabeled *m*-tyrosine was eluted with 3 N NH_4OH (2 ml).

(*c*) *Oxidation of* D-m-*tyrosine to* m-*hydroxyphenylpyruvic acid.* The complete reaction mixture contained the following components (in μmoles) in a final volume of 1.0 ml: sodium pyrophosphate, pH 8.3, 100; FAD, 0.1; D-[2-^{14}C]*m*-tyrosine, 1.0 (containing 85,000 cpm); and 0.25 ml of tissue homogenate. Boiled homogenates served as controls. After incubation, the product was isolated as described in (a).

(*d*) *Decarboxylation of* L-m-*tyrosine to* m-*tyramine.* The complete reaction mixture contained the following

components (in μmoles) in a final volume of 1.0 ml: potassium phosphate, pH 7.2, 100; Catron (monoamine oxidase inhibitor), 0.01; L-[2-^{14}C]-*m*-tyrosine, 3.0 (containing 218,000 cpm); and 0.5 ml of tissue homogenate. Boiled homogenates served as controls. After incubation, the reaction was stopped by placing the samples in a boiling water bath for 2 min. The precipitate was removed by centrifugation, and 0.5 ml of the supernatant fraction was applied to an Amberlite CG-50 column. The column was washed with water (5 ml), and radiolabeled *m*-tyramine was eluted with 2 N HCl (2 ml).

(*e*) *Oxidative deamination of* m-*tyramine and* m-*octopamine.* The complete reaction mixture contained the following components (in μmoles) in a final volume of 1.0 ml: potassium phosphate, pH 7.5, 10; [1-^{14}C]*m*-tyramine or [1-^{14}C]*m*-octopamine, 1.0 (containing 100,000 cpm); and 0.5 ml of tissue homogenate. Boiled homogenates served as controls. After incubation, the reaction was stopped by placing the samples in a boiling water bath for 2 min. The precipitate was removed by centrifugation, and the supernatant fraction was applied to an Amberlite CG-50 column. The product was eluted with water (2 ml).

RESULTS

Urinary metabolites of [2-^{14}C]m-*tyrosine*

Three hr after the rats were given DL-[2-^{14}C]*m*-tyrosine (10–300 mg/kg, 4.5 × 10^6 dis./min) intraperitoneal injections, about 70 per cent of the radioactivity initially administered was recovered from the urine. The amount recovered from the urine over a period of 24 hr was about 85 per cent and the urinary metabolites of the amino acid were usually analyzed from the 24-hr samples. A typical chromatographic elution pattern of the urinary metabolites of DL-*m*-tyrosine from the amino acid analyzer is shown in Fig. 1. Seven radioactive components were detected. The identities of the metabolites were confirmed by co-chromatography with authentic compounds on thin-layer plates (see Materials and Methods). About 85 per cent of the urinary radioactivity was accounted for as *m*-hydroxyphenylpyruvate, *m*-hydroxyphenylacetate, *m*-tyramine and unchanged *m*-tyrosine. It was observed that the proportion of these metabolites varied slightly, depending on the amount of DL-*m*-tyrosine administered. When the dosage of the amino acid was increased from 10 to 300 mg/kg, there was a decrease in the ratio of the acidic metabolites/*m*-tyramine (Table 3).

Table 3. Major metabolites of DL[2-^{14}C]*m*-tyrosine in 24 hr urine*

Metabolites	Per cent of urinary radioactivity		
	DL-*m*-tyrosine (mg/kg)		
	10	100	300
m-Hydroxyphenylpyruvate	13.5	8.9	8.1
m-Hydroxyphenylacetate	30.4	29.0	25.0
m-Tyrosine	14.0	17.5	17.6
m-Tyramine	26.8	31.2	36.0

* DL-[2-^{14}C]*m*-tyrosine (containing a constant amount of radioactivity, 4.5 × 10^6 dis./min) was administered by intraperitoneal injection. Urine was collected for 24 hr and the radioactive metabolites were identified and quantitated, as described in Materials and Methods.

Conjugates. There were two radioactive peaks (35 min and 158 min, Fig. 1) emerging from the column which did not correspond to the elution times of the available *m*-hydroxyphenyl compounds. However,

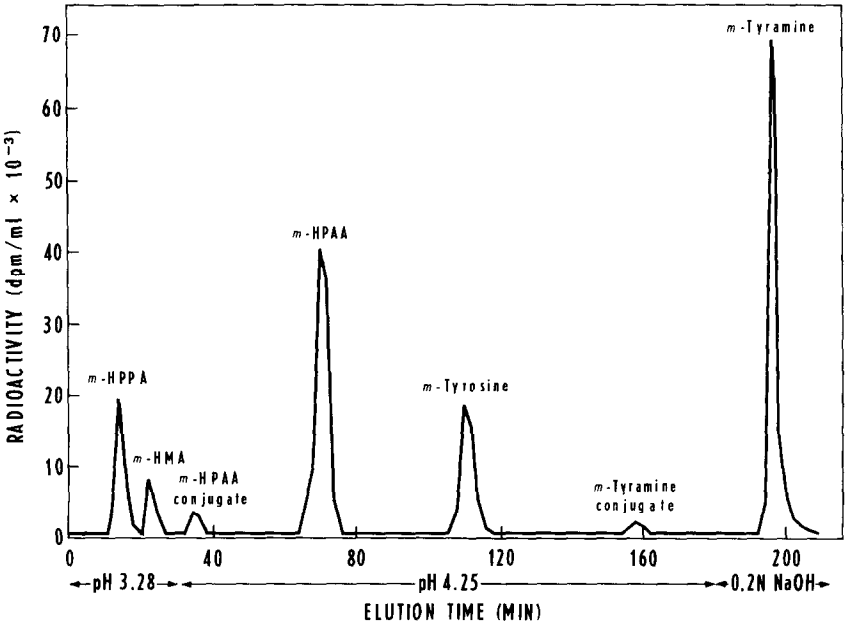

Fig. 1. Chromatographic elution pattern of urinary metabolites of DL-[2-^{14}C]*m*-tyrosine from the amino acid analyzer. Elution conditions were as described in Table 1.

Table 4. Metabolites of D-, L- and DL-[2-^{14}C]*m*-tyrosine in 24 hr urine after intraperitoneal administration (100 mg/kg) to rats*

Metabolites	Per cent of urinary radioactivity		
	D-*m*-Tyrosine	L-*m*-Tyrosine	DL-*m*-Tyrosine
m-Hydroxyphenylpyruvate	6.2	11.3	8.9
m-Hydroxymandelate	5.2	7.3	3.4
Conjugate of *m*-hydroxyphenylacetate	2.3	12.2	3.5
m-Hydroxyphenylacetate	16.7	57.3	29.0
m-Tyrosine	26.1	0.0	17.5
Conjugate of *m*-tyramine	0.0	8.4	6.8
m-Tyramine	40.5	3.3	31.2

* Twenty-four h after the administration D-, L- or DL-[2-^{14}C]*m*-tyrosine (4.5×10^6 dis./min), about 85 per cent of the injected radioactivity was recovered from the urine. Identification and quantitation of the radioactive metabolites were as described in Materials and Methods.

upon hydrolysis in 6 N HCl, the 35-min peak yielded labeled *m*-hydroxyphenylacetic acid and the 158-min peak yielded *m*-tyramine, as judged by co-chromatography on the analyzer and thin-layer plates. They were identified, therefore, as conjugates of *m*-hydroxyphenylacetic acid and *m*-tyramine. Furthermore, tests for the phenolic functional group (by ultraviolet spectroscopy and color reaction with diazotized *p*-nitroaniline) of the unknown compounds before and after acid hydrolysis revealed that the conjugate of *m*-hydroxyphenylacetic acid had a free phenolic group while that of the *m*-tyramine conjugate was blocked.

Metabolites of D- *and* L-[2-^{14}C]m-*tyrosine.* We have reported earlier that L-M-tyrosine was more potent than the D-isomer in depleting brain biogenic amines [26]. It was of interest, therefore, to compare the metabolism of the optical isomers of *m*-tyrosine. The results are shown in Table 4. It was observed that the proportion of acidic metabolites of *m*-tyrosine was about 88 per cent for the L-isomer and 30 per cent for the D-isomer. A considerable proportion (26 per cent) of D-*m*-tyrosine was excreted unchanged. It is noteworthy that, although *m*-tyramine was a major metabolite of D-*m*-tyrosine (40 per cent vs 3 per cent from the L-isomer), none of it was observed in conjugated form.

Metabolism of ^{14}C-*labeled* m-*hydroxyphenyl acids and amines.* In order to explore the relationships of the *m*-tyrosine metabolites, the metabolism of some *m*-hydroxyphenyl compounds was studied. As shown in Table 5, a major metabolic pathway for *m*-tyramine and *m*-octopamine was their oxidation to *m*-hydroxyphenylacetic acid and *m*-hydroxymandelic acid which were then excreted without further metabolism. The appearance of *m*-tyrosine and *m*-tyramine after the administration of *m*-hydroxyphenylpyruvic acid suggested that a portion of the keto acid was transaminated, giving rise to the amino acid and its decarboxylation product.

Effect of dopa decarboxylase inhibitor on the metabolism of [2-^{14}C]m-*tyrosine and* [2-^{14}C]m-*hydroxyphenylpyruvic acid.* In order to determine whether decarboxylation is the major pathway for the metabolism of *m*-tyrosine [27], the effect of the dopa decarboxylase inhibitor NSD-1034 [28] was investigated (Table 6). When animals were pretreated with NSD-1034 (100 mg/kg), over 80 per cent of the injected *m*-tyrosine was excreted unchanged. The conversion of *m*-hydroxyphenylpyruvic acid to *m*-tyrosine also became more evident. Incidentally, a small yet significant amount (0.6 per cent) of radioactive dopa was observed among the urinary metabolites of [2-

Table 5. Major urinary metabolites of [^{14}C]*m*-hydroxyphenyl acids and amines*

Metabolites	Per cent of urinary radioactivity				
	m-HPPA	*m*-HMA	*m*-HPPA	*m*-Tyramine	*m*-Octopamine
m-Hydroxyphenylpyruvate	32				
m-Hydroxymandelate		98		16	91
m-Hydroxyphenylacetate	26		85	68	
m-Tyrosine	1				
m-Tyramine	34			5	

* [^{14}C]*m*-hydroxyphenyl compounds were administered by intraperitoneal injections. The dosage injected was 100 mg/kg (4.5 to 5.7 $\times 10^6$ dis./min) for the acids and 40 mg/kg (4.0 to 5.5 $\times 10^6$ dis./min) for the amines. Urine was collected for 24 hr and about 85 per cent of the injected radioactivity was recovered. The radioactive metabolites were identified and quantitated as described in Materials and Methods. When the amount of metabolites was < 0.2 per cent of the total urinary radioactivity, the values were not given in the table.

Table 6. Effect of dopa decarboxylase inhibitor on the metabolism of DL-*m*-tyrosine and *m*-hydroxyphenylpyruvate*

Metabolites	Per cent of urinary radioactivity			
	m-Tyrosine	NSD-1034 + *m*-tyrosine	*m*-HPPA	NSD-1034 + *m*-HPPA
m-Hydroxyphenylpyruvate	9	8	32	39
m-Hydroxymandelate	3			
m-Hydroxyphenylacetate	29	3	26	29
m-Tyrosine	18	82	1	18
m-Tyramine	31	6	34	7

* Dopa decarboxylase inhibitor NSD-1034 (100 mg/kg) was injected intraperitoneally into rats. Control animals received saline. DL-[2-^{14}C]*m*-tyrosine (100/mg/kg) or [2-^{14}C]*m*-hydroxyphenylpyruvic acid (100 mg/kg) was administered similarly 30 min later. Urine was collected for 24 hr and the radioactive metabolites were identified and quantitated, as described in Materials and Methods. When the amount of metabolite was < 0.2 per cent of the total urinary radioactivity, the values were not given in the table.

^{14}C]*m*-tyrosine in animals pretreated with NSD-1034. The *in vivo* conversion of *m*-tyrosine to dopa was reported in an earlier publication [20].

Tissue distribution of DL-[2-^{14}C]m-*tyrosine after intraperitoneal administration*

Fifteen min after the administration of DL-[2-^{14}C]*m*-tyrosine (100 mg/kg), about 5–7 per cent of the radioactivity could be recovered from the six organs examined. Thereafter the radioactivity declined, and at 60 min only 1–2 per cent was present in these organs. The amount of radioactivity accumulated/organ at 15 min was found to be in the following order: kidneys (3 per cent) $\simeq$ liver (3 per cent) > brain (0.4 per cent) > spleen (0.2 per cent) $\simeq$ heart (0.2 per cent) > adrenals (0.07 per cent). These values were based on the radioactivity initially administered (4.5×10^6 dis./min = 100 per cent).

Metabolism of m-*hydroxyphenyl compounds* in vitro

In vitro experiments were performed with tissue homogenates in order to demonstrate the origins of the major metabolites of [2-^{14}C]*m*-tyrosine. Brain, kidney and liver were chosen for these studies since they represented over 90 per cent of the radioactivity accumulated in the organs examined. The results are summarized in Table 7. It was observed that the enzyme activities in the brain, with the exception of the oxidative deamination of *m*-hydroxyphenyl amines, were considerably lower than those of kidney or liver when assayed under identical conditions. The highest activity for the oxidation of D-*m*-tyrosine was found in the kidney where D-amino acid oxidase is mainly localized [29].

DISCUSSION

In the present experiments, the metabolism of [2-^{14}C]*m*-tyrosine *in vivo* was studied. The results obtained indicate that *m*-tyrosine, administered by intraperitoneal injection, was eliminated mainly through urinary excretion. The major metabolites identified were consistent with results reported from *in vitro* experiments that *m*-tyrosine could be transaminated to form *m*-hydroxyphenylpyruvic acid [18], decarboxylated to *m*-tyramine [17], and that *m*-tyramine could be oxidized to form *m*-hydroxyphenylacetic acid [15]. Conjugates of *m*-hydroxyphenylacetic acid and *m*-tyramine (labile to hot acid) were also identified among the urinary metabolites (Fig. 1). The nature of the conjugates is not clear at this stage. Possible candidates are *m*-hydroxyphenylacetylglutamine [30, 31], *m*-hydroxyphenylacetylglycine [27, 32–34], and *m*-tyramine *o*-sulfate ester [35, 36].

Although most of the results obtained from the pharmacological studies on *m*-tyrosine were carried out with the racemate, it is well known that the enantiomers

Table 7. Metabolism of *m*-hydroxyphenyl compounds *in vitro**

Substrate	Product	Product formed (nmoles/min/g tissue)		
		Brain	Kidney	Liver
L-*m*-Tyrosine	*m*-HPPA	8.8	39.7	61.0
D-*m*-Tyrosine	*m*-HPPA	2.6	124.5	18.0
m-HPPA	*m*-Tyrosine	20.2	127.8	144.1
L-*m*-Tyrosine	*m*-Tyramine	7.3	395.8	441.7
m-Tyramine	*m*-HPAA	54.3	29.1	148.2
m-Octopamine	*m*-HMA	16.1	8.5	45.0

* Assay conditions were as described in Materials and Methods.

of aromatic amino acids are not biologically equivalent. Experiments on the metabolism of the D- and L-isomers of [2-^{14}C]*m*-tyrosine revealed that there were marked differences in the fates of the two isomers (Table 4). The observation that *m*-tyramine was a major metabolite of D-*m*-tyrosine and yet none of it appeared in the conjugated form is reminiscent of that reported on the metabolism of D-dopa by Shindo and Maeda [37], and Sourkes *et al.* [38]. A possible explanation for the metabolism of D-*m*-tyrosine is its oxidation in the kidney by D-amino acid oxidase to the keto analogue, coupled with transamination of the product to form L-*m*-tyrosine. The L-amino acid is then decarboxylated to *m*-tyramine which is excreted without further metabolism. The high activity of D-amino acid oxidase observed in the kidney (Table 7) and the conversion of *m*-hydroxyphenylpyruvic acid to *m*-tyrosine *in vivo* and *in vitro* (Tables 6 and 7) give support to the above hypothesis.

In order to establish the metabolic pathway of *m*-tyrosine, the metabolism of some *m*-hydroxyphenyl compounds was investigated both *in vivo* and *in vitro* (Tables 5–7). When the decarboxylation of *m*-tyrosine was inhibited by NSD-1034, over 80 per cent of the amino acid was excreted unchanged. This is taken as evidence that the administered *m*-tyrosine is metabolized almost exclusively by the pathway through *m*-tyramine to give *m*-hydroxyphenylacetic acid. Results from the experiments with liver, kidney and brain homogenates showed that all three organs have the enzymes necessary to catalyze the transamination and decarboxylation of L-*m*-tyrosine, the oxidation of D-*m*-tyrosine, the transamination of *m*-hydroxyphenylpyruvate, and the oxidative deamination of *m*-tyramine and *m*-octopamine. Although the conversion of *m*-tyramine to *m*-octopamine was not detected in the crude homogenates, we were able to demonstrate the reaction with a partially purified preparation of dopamine-β-hydroxylase from rat brain (data not shown). This is in agreement with the results obtained by Creveling *et al.* [16]. Therefore, a possible pathway for the appearance of *m*-hydroxymandelate in rat urine is via the β-hydroxylation of *m*-tyramine to *m*-octopamine, which is then acted upon by monoamine oxidase. A summary of the major metabolic pathways of *m*-tyrosine in the rat is given in Fig. 2.

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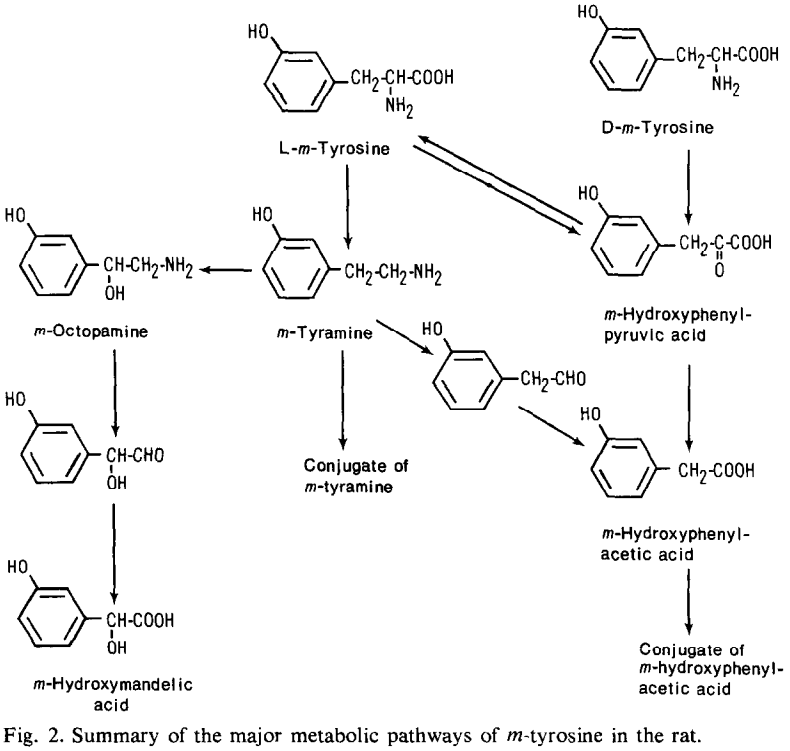

Fig. 2. Summary of the major metabolic pathways of *m*-tyrosine in the rat.

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